Karyology study of six cytotypes of *Vicia* L. in Tehran Province (Iran)

Abstract

The genus *Vicia* L. is an important forage crop and consists of approximately 160 annual and perennial species. Analysis performed on six species of this genus. The basic chromosome number varied x=5, x=6 and x=7. According to Stebbins classification, species were classified as symmetric class of 3A. Based on intra chromosomal symmetry, *V. villosa* Roth. had the most asymmetrical and evolutionary karyotype. *V. cordata* (Wulf. ex Hoppe) Arcangeli, Comp. had the most symmetrical karyotype. The highest distance was observed between these two species using cluster analysis.

Keywords: Chromosome, Cytology, Fabaceae, Vetch

1. INTRODUCTION

Fabaceae is one of the third-largest family of flowering plants. It has many agriculturally genus. It includes trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume). The family is widely distributed, and is the third-largest land plant family in number of species. Vetch (*Vicia* Linnaeus, 1753) with more than 160 species, located in Europe, Asia and North America, in the temperate regions of South America and Tropical Africa [1,2,3,4].

Mediterranean region is ~~the main~~ center of variability of the genus *Vicia* L. [5]. The genus is characterized by ~~having~~ a style pubescent all around, or bear on the adaxial side, never twisted and stem non winged. Many species such as *V. sativa* L., *V. cracca* L. and *V. narbonensis* L. have high protein fodder plants for several animals. Also they play an important role in environment of soil, increasing the nutritive value of drought-resistant pasture [6]. This genus has been subdivided into three or four major groups recognized as different levels. Kupicha in 1976, classified this genus into two subgenera: *Vicilla* (Schur.) Rouy (including 17 sections) and *Vicia* (including five sections). The basic chromosome numbers reported in *Vicia* are x=5, 6 and 7. Most of the species studied are diploids with 2n=2x= 10, 12, 14; however a number of tetra- and hexaploids were also reported [7,8,9,10]. The speciation in this genus is accompanying by variation in chromosome size and hybridization is common in the genus [11]. The kayotypic variability between *Vicia* species, which makes the genus an interesting model for the study of plant genome and karyotype evolution [7,12,13]. The present study considers a mitotic analysis of six species of *Vicia* selected from Tehran province in Iran and try to reveal the chromosome numbers, shape, size and karyotypic evolution to determine the best taxa for crop breeding and marketing programs in this genus.

MATERIAL AND METHODS

Plant and seed materials were collected from natural habits (Table 1). Seeds of six *Vicia* species including *V. sativa*; *V. ervilia*, *V. hyrcanica* , *V. villosa* Roth, *V. cordata* L. and *V. peregrina* L. were germinated on wet filter paper in Petri dishes and left at 22° C temperature for three days. Root tip meristems

obtained from seedling were pretreated in 8- hydroxyl- quinalin (2 Mm) at 4° C for five h, fixed in 1:1 (v/v) solution of formalin 10٪and chromic acid 1٪for 24 h at 4°C, then root tips were rinsed for 3 h in distilled water and stored in 70٪ ethanol at 4°c. For hydrolyzing, the root tips were treated NaOH 1N for 10 min at 60°C and stained with aceto-iron-hematoxilin solution for 4 h at 30°C. After each step, root tips were washed briefly in distilled water. Meristematic region with 1 mm of length excised and macerated in cytase enzyme at room temperature for 1 h. Squash preparations on slids were made in 45٪ acetic acid [13]. Chromosome measuremants including long arm (LA), short arm (SA), total length of chromosomeset (TL), arm ratio (AR) and centromic index (CI) were made from 15 and 10 enlarged well-spread methaphases, for karyotype analysis in each species, using Micromeasure soft-ware developed by the Biology Department State University, USA, available on Internet at http://www. Colostateedu/Depts/Biology/Micromeasure. Karyotype asymmetry was estimated by three different methods namely, total form percentage (TF٪) [∑S/∑TL) × 100]; difference of relative length (DRL) [Max RL%- Min RL%]; intrachromosomal asymmetry index (A1) [1- ∑(S/l)/n] and interchromosomal asymmetry index (A2) [Sd/x]. Both indices (A1 and A2) are independent to chromosome number and size. Also karyotype evolution has been determined using the symmetry classes of Stebbins [15]. Karyotype formula was determined by chromosome morphology based on centrometer position according to classification of Levan [16]. Karyograms were drawn based on length of chromosome size. Clustering was performed using UPGMA method to examine karyotype similarity among species.

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Table1- Populations and their localities

Population	Locality
V. sativa	Tehran: Karaj, 1350 m
V. cordata	Tehran: Damavand, 2100 m
T. villosa	Tehran: Lavasanat, 1400 m
T. hyrcanica	Tehran: Damavand,1000 m
T. peregrina	Tehran: Firoozkoh road, 2000 m
T. ervilia	Tehran: Sohanak , 2010 m

RESULTS AND DISCUSSION

Karyological data

The results showed that all of examined species are diploid and the basic chromosome numbers are x= 5, x=6 and x=7. The karyotypes of species are illustrated in Table 2. The mean value of long arm was varied from 3.60 µm in *V. ervilia* to 5.42 µm in *V. villosa*. The average of short ~~are~~ was different from 1.95 µm in *V. ervilia* to 3.16 µm in *V. cordata*. The mean value of total length of chromosome was varied from 34.52 µm in *V. cordata* to 52.76 µm in *V. villosa* and finally the mean value of arm ratio was changing from 1.15 µm in *V. cordata* to 2.56 in *V. villosa*. The chromosome in these species were mostly submetacentric to metacentric, in such as manner that chromosomes in *V. cordata* were metacentric and in other species were composing of metacentric and submetacentric. Symmetry types of Stebbin ~~are~~ given in Tab 2. In terms of the Stebbins system, the karyotype of species mostly sizes 3A class, which are considered majorly primitive classes in this system. Intrachromosomal

asymmetry index (A1) expresses the arm ratio of each pair of homologous chromosomes. The interchromosomal asymmetry index (A2) corresponds to Pearson's coefficient of dispersion and gives an idea of the asymmetry caused by the different length of the chromosomes. By using the Romero-Zarco, asymmetric indices of A1 and A2, we can determine the more asymmetric karyotype among the species which have the Stebbins classes of symmetry. In *V. cordata* possesses the lowest A1 value (0.83) and that DRL value was 8.28, therefore has a more symmetric karyotype and *V. villosa* possessed the highest A1 value (0.95), so has a more asymmetric karyotype. These results also showed the lowest value of A2 in range 0.36 – 0.64 and the highest value of TF% ranged from 28.01 to 45.85. In general, on intrachromosomal symmetry (A1 and TF%), *V. villosa* karyotype had the most asymmetrical and evolutionary and *V. cordata* had the most symmetrical karyotype in these six species. According to interchromosomal asymmetry (A1 and DRL), *V. villosa* had the most asymmetrical karyotype in all of these species (Table 2). Asymmetry index TF% ranged from 28.01 to 45. 85. Grouping of the species are studied based on their relative karyotypic as well as mitotic characterestics (Tabel 2; Figure 1,2).

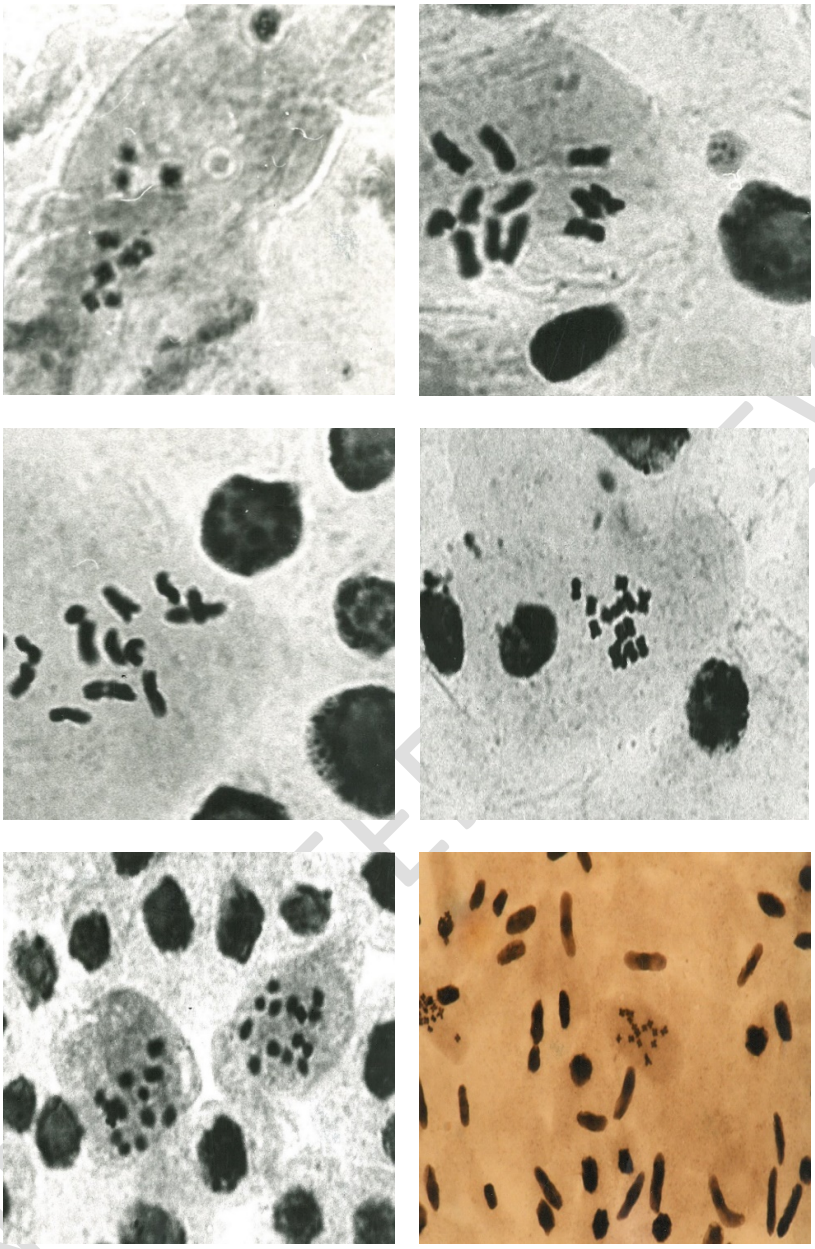


Figure 1- Mitotic metaphases of six *Vicia* species. Bars = 5µm.

1a- *V. cordata*, 1b- *V. peregrina*, 1c- *V. vilosa*, 1d- *V. sativa*, 1e- *V. ervilia*, 1f- *V. hyrcanica*

Table 2- Karyotype characteristics of six *Vicia* species

Species	2n	LA	SA	TL	AR	r - value	CI	DRL	TF%	S%	A1	A2	KF
V. cor	10	3.73	3.16	34.52	1.18	0.84	2.27	8.28	45.85	60.62	0.83	0.63	5m
V. vil	14	5.42	2.11	52.76	2.56	0.38	1.86	11.5	28.01	25.3	0.95	0.36	1m+2sm+4st
V. hyr	12	4.32	2.18	39.04	1.98	0.5	1.96	8.72	33.52	29.92	0.92	0.45	1m+4sm+1st
V. per	12	4.58	3.06	45.96	1.49	0.66	2.4	9.38	40.03	36.37	0.89	0.39	4m+2sm
V. sat	14	4.05	2.4	45.23	1.68	0.59	2.54	9.86	37.23	42.03	0.92	0.64	4m+2sm+1st
V. erv	14	3.6	1.95	38.95	1.84	0.54	2.54	8.91	35.12	20.38	0.93	0.54	2m+5sm

Abbriviation: V. cor= *V. cordata*; V. hyr= *V. hyrcanica*; V. per= *V. peregrina*; V. sat= *V. sativa*; V. erv= *V. ervilia*

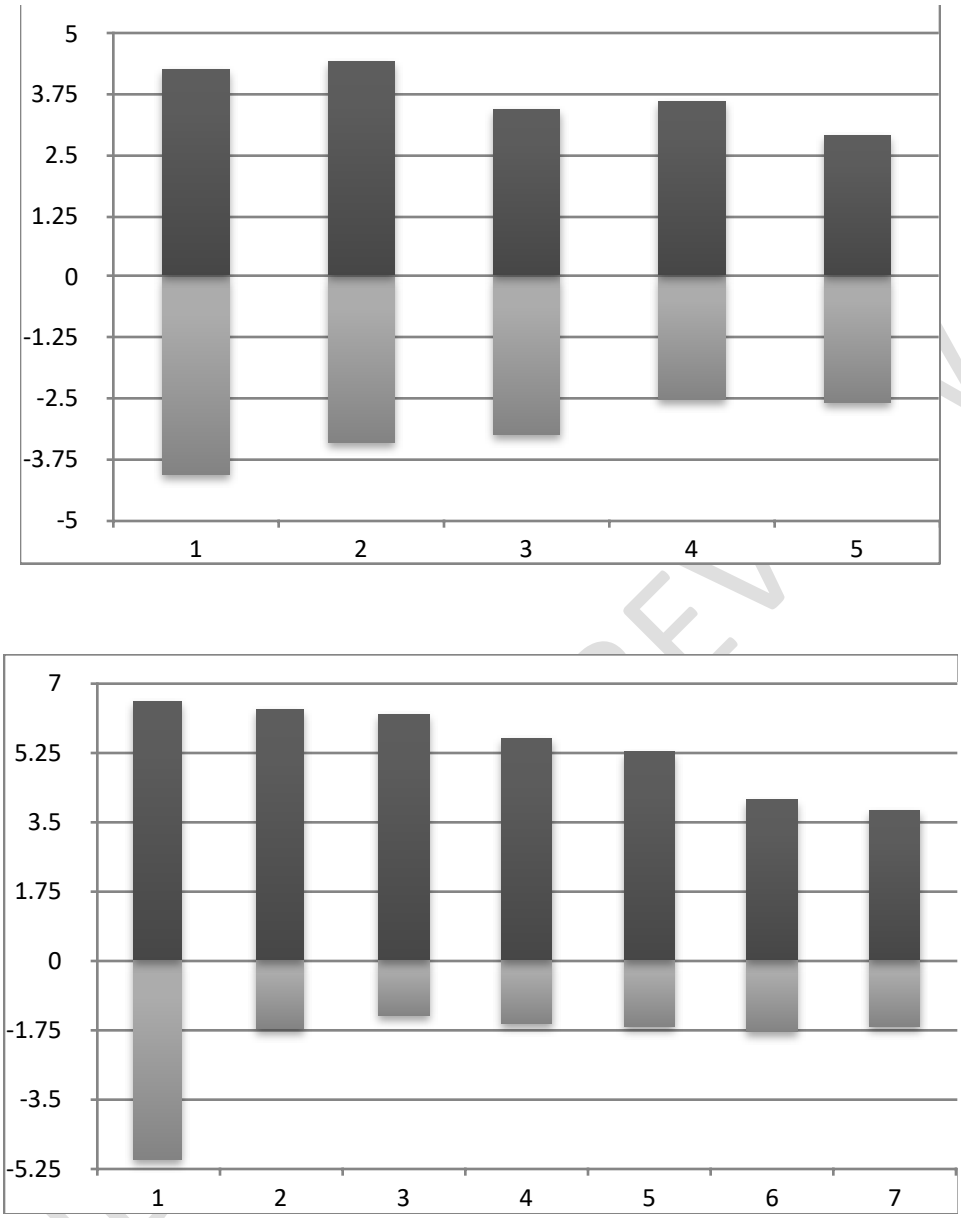
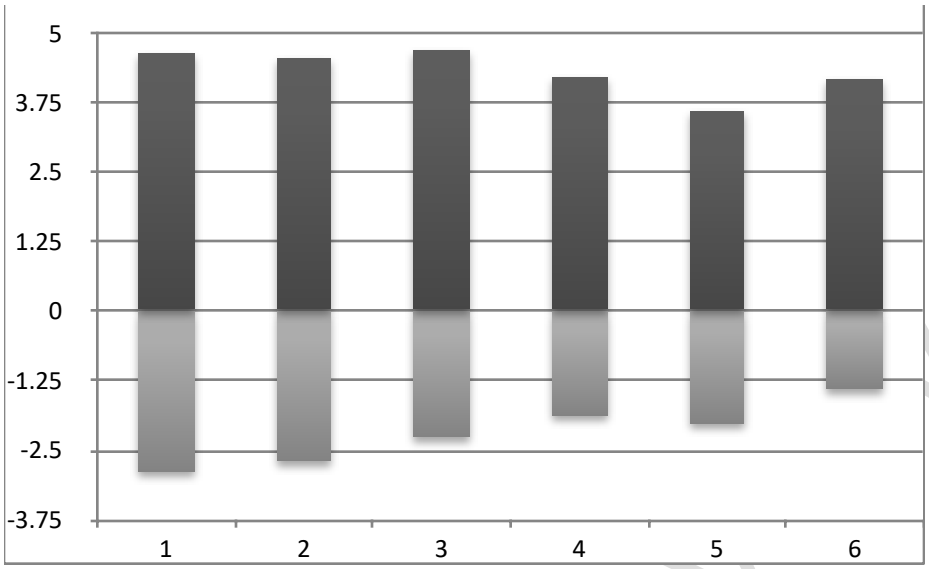


Figure 2- Karyogram of six *Vicia* species. 1a- *V. cordata*, 1b- *V. villosa*, 1c- *V. hyrcanica*, 1d- *V. peregrina*, 1e- *V. sativa*, 1f- *V. ervilia*



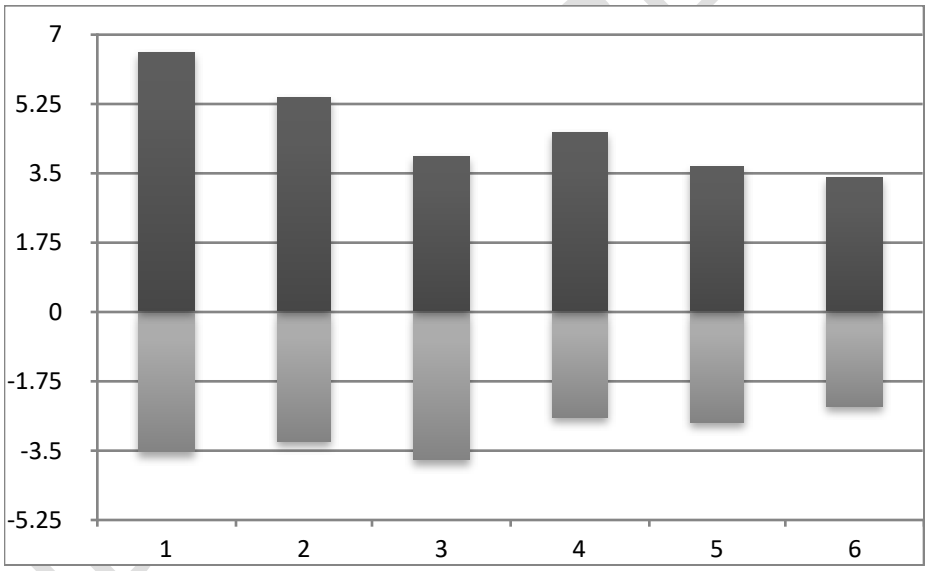
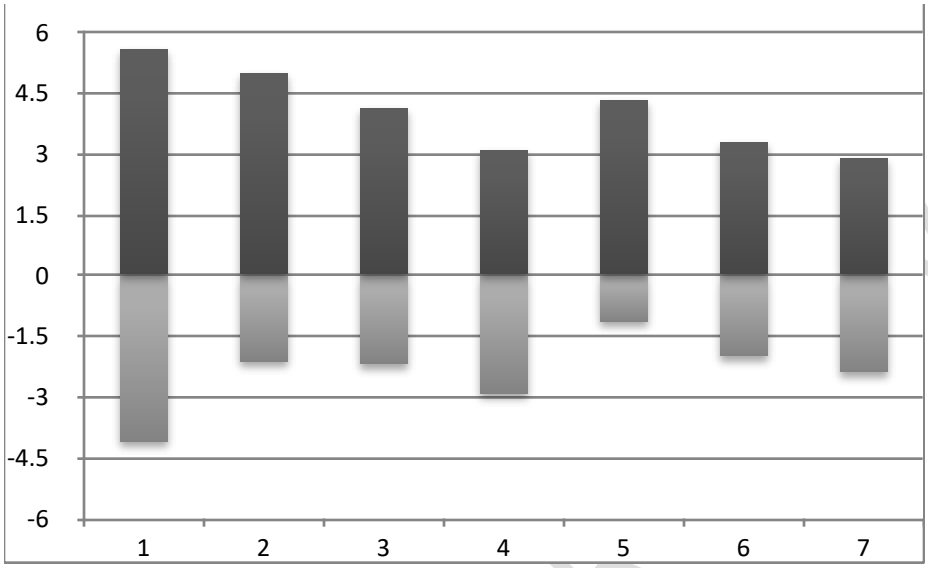


Figure 2- continued



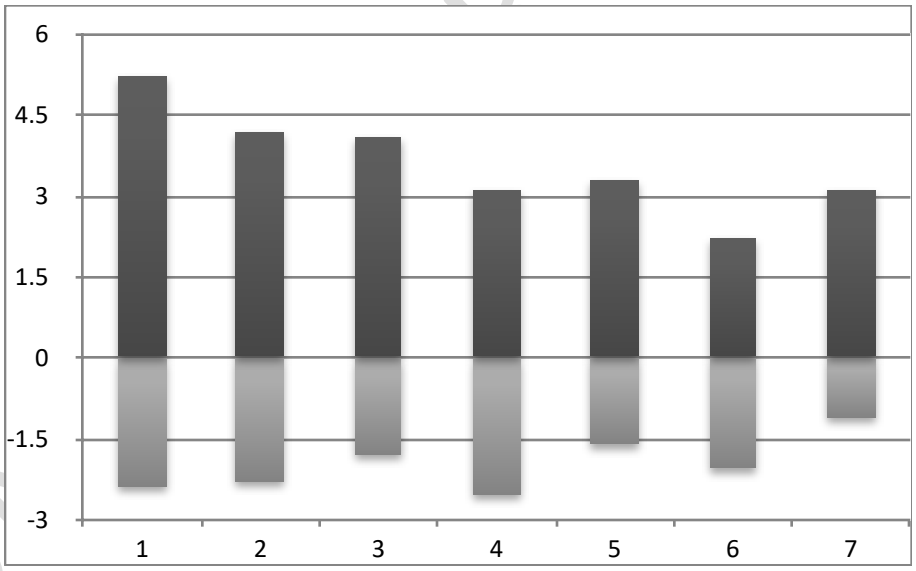


Figure 2- continued

Cluster analysis

Cluster analysis using by the average linkage method classified the species into two main groups. The highest distance was obtained between *V. cordata* and *V. villosa* and the lowest distance was obtained between *V. hyrcanica* and *V. villosa* (Figure 3).

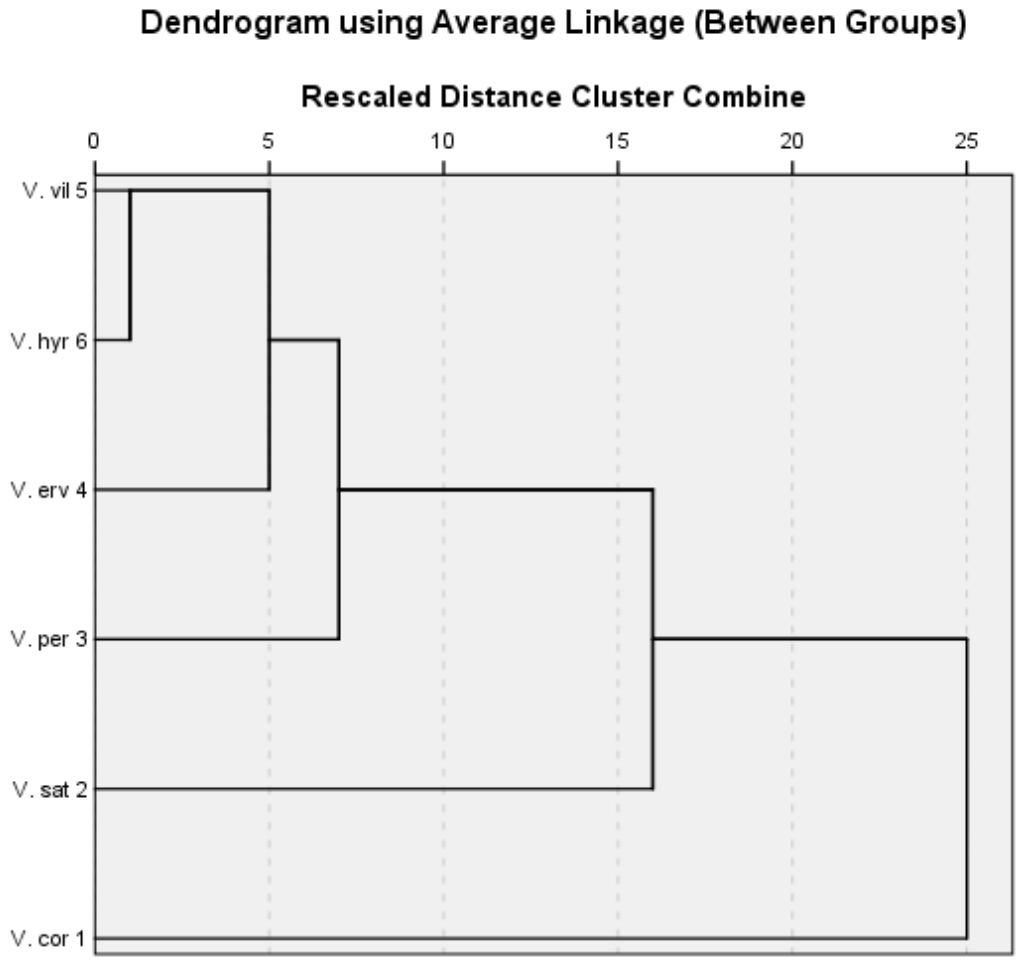


Figure 3- Dendrogram of six species of *Vicia* by analyzing 13 karyotypic parameters using average linkage cluster analysis method: 1- *V. cordata*; 2- *V. sativa*; 3- *V. peregrina*; 4- *V. ervilia*; 5- *V. hycanica*; 6- *V. villosa*

Goldblatt (1981) suggested x=14 as the basic number for the subfamily Faboideae, x=7 for the tribe *Vicieae* and x=6, x=7 for the genus *Vicia* [17]. It showed that in forage legumes the main basic chromosome is (x=5 to x=8). Numerous reports have shown that the most frequent basic chromosome number for *Vicia* genus are x=5, x=6 and x=7.[18,4,6,11, 12,17,18]. Most of species in this genus are diploids while only six of them are polyploids. Analysis of karyotype formula showed that, generally, the number of "sm" chromosomes was more than "m" chromosomes except for *V. cordata* and *V. sativa*. Hanlet and Mettin (1989) reported that meta and submetacentric chromosomes are the dominant chromosomes forms in the subgenus *Vicilla*. Also, our result is agreement with Gaffarzadeh *et al*. about other species in this genus [20]. *V. villosa* had the highest A1 value, exhibiting the most asymmetrical and intrachromosomally derived karyotype, while *V. cordata* was introduced as the most symmetrical karyotype (Table 1). In view of the fact that, fewer DRL value illustrated more symmetry of karyotype. *V. villosa* and *V. cordata* respectively with DRL 11.51 and 8.28 values had the most asymmetry and symmetry karyotypes, respectively. Similarly, high DRL value leads to more changes in the construction of chromosomes. Plitman, 1967; Schaffer, 1973, noted that speciation process in *Vicia* is accompanied by karyotype differentiation of chromosome number [21,22]. Chromosome changes may have contributed towards the erection of interspesific hybridization barriers in this plant group [23,24,25]. Grouping based on karyotypic parameters indicated that *V. cordata* was located far from other species, specially from *V.villosa* and *V. hyrcanica*. This study based on cytological data showed that the species with the lowest metric distance, may lead us to use species in crosses for including the highest genetic variations (Figure 3).

Conclusion

The present study showed the change in the chromosomal traits as one of as the mechanism of inter and intra species diversification the *Vicia* genus as well as the earlier cytological reports. The differences in karyotype formula and asymmetric indices found among the species suggest that structural changes of chromosomes may contribute to the diversification of the genus. These genomic differences ~~could~~ be used for breeding purposes. I can

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